The Early Development of Chrotogonus trachypterus Blanchard. (A Major Pest of Cotton-Seedlings) (Orthoptera : Acrididae)

Dissertation submitted for the award of the
Degree of Master of Philosophy
IN
ZOOLOGY

By
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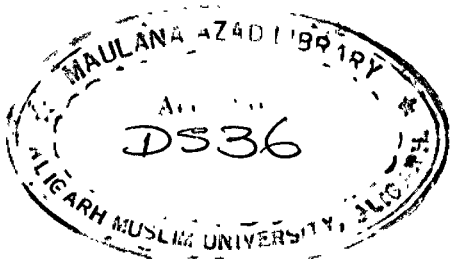
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THE EARLY DEVELOPMENT OF *CHROTOGONUS TRACHYPTERUS* BLANCHARD.

(A MAJOR PEST OF COTTON-SEEDLINGS)

(ORTHOPTERA : ACRIDIDAE)

A Dissertation

Submitted to the

Department of Zoology

Aligarh Muslim University, Aligarh

By

MANZOOR A. SIDDIQUI

In Partial Fulfillment of the

Requirements for the Degree of

MASTER OF PHILOSOPHY

April, 1975.

CERTIFICATE

Certified that this dissertation submitted by Mr. Manzoor A. Siddiqui is his original work on *Chrotogonus trachypterus* Blanchard. carried out under my supervision. This fulfills the partial requirements of the M. Phil. degree.

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C O N T E N T S

I. ACKNOWLEDGEMENTS

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I am also indebted to Prof. Shah Mashhood Alam, Head, Department of Zoology, Aligarh Muslim University, Aligarh for providing me necessary laboratory facilities for this research work.

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II. INTRODUCTION

The grasshoppers are admirable material for biological investigations as they are available in abundance, small in size and hardy. Grasshopper eggs have been used extensively in developmental and experimental embryology. Considerable work has been done on several orders of insects, like Coleoptera, Hemiptera, Lepidoptera and Diptera but the insects belonging to the order Orthoptera, particularly from the family Acrididae have received little attention. Mostly the large-sized grasshoppers and locusts have been the subject of wide investigations. Small-sized grasshoppers, despite their harmfull activities, have not received due attention in respect of their embryonic development.

The present work was undertaken with a view to provide a reasonably comprehensive account of the early embryology of Chrotogonus trachypterus Blanch. This species which has been identified by Dr. Mittal was selected for study due to its great economic importance. In North India particularly in "Uttar Pradesh" it is abundantly found during the months of August and September. Its adults as well as nymphal stages are a constant source of damage to cotton at the seedling stage. Besides the cotton seedlings, it may also be considered as a sporadic pest of cabbage, cauliflower and castor plants.

Chrotogonus completes its development in about two weeks under favourable conditions of temperature and humidity. At room temperature this period may be considerably extended as the eggs undergo diapause - a phenomenon much in evidence in this group of insects.

III. REVIEW OF LITERATURE

The literature so far available provides only an incomplete picture of the embryonic development of grasshoppers. An early work in this connection is that of Mc-Clung (1905) and Mc-Nabb (1928). The former has studied the behaviour of chromosomes in Orthopteran spermatogenesis, and the later has given a comparative account of meiosis, fertilization and cleavage in Crotophaga viridifasciata and Circotettix verruculatus. They have paid special attention to the behaviour of chromosomes during the early development of the egg.

Another important work in this connection is that of Slifer (1930-1958). She has worked on a variety of subjects concerning insect development like mitotic activity in the grasshopper embryo, blastokinesis in the living grasshopper egg and external morphology of grasshopper embryos of known age with a known temperature history. Besides this, she has also worked on different experimental aspects of the developing egg and the adult grasshoppers. King & Slifer (1934) described the early embryonic development of Melanoplus differentialis upto the formation of early cleavage. Roonwal (1933-1954) however, has given a fairly good account of the embryology of the African migratory locust, Locusta migratoria migratorioides.

Jhingran (1949) has described early embryology of the desert locust, Schistocerca gregaria. Manna (1954) has confined his study on the number of chromosomes during mieosis in fifteen species of Indian grasshoppers. Nath and Gupta (1960) have restricted their studies on the histochemistry and physiology of spermatogenesis of Chrotogonus trachypterus Blanch. Hunter (1964) has studied egg development in desert locust, Schistocerca gregaria Forskal, in relation to the availability of water. Sharan (1958-1972) has confined his studies on the formation of provisional embryonic cuticle development of the compound eye and the development of the alimentary canal in Locustana pardalina Walker.

IV. MATERIAL AND METHODS

Chrotogonus trachypterus Blanch. (Orthoptera, Acrididae) is available throughout the year in the vicinity of Aligarh. It is abundantly found during the month of August and September. Mature females were collected with few males in the middle and later part of September from the Aligarh Fort area and near the University Agriculture Farm where the vegetable crops like cabbage, cauliflower were cultivated. They were reared in the laboratory under controlled condition of temperature and humidity, keeping the temperature at 32°C and 100% R.H. and were fed on cotton seedlings grown in experimental plots. In the absence of cotton seedlings, which constitute its primary food, the grasshoppers were fed on castor and cauliflower leaves.

For the collection of eggs, Chrotogonus females, together with few males were kept in cages supplied with metal tubes containing damp sand which serves as an ideal site for oviposition under captivity. Immediately after the completion of the oviposition the egg pods were labelled and transferred to a petridish containing moistened sand. These dishes were put in an incubator regulated at 32°C. After desired time intervals the eggs were removed from the pod and fixed immediately.

Various fixatives like Carnoy, Gilson, Zenker's fluid and Petrunkewitch were tried over the material but none did give any satisfactory result. Results obtained with hot alcoholic Bouins however, were considered best and this fixative was therefore used throughout the study. The eggs were punctured at the anterior end or were cut into two equal half after placing them for a while in the fixative thus avoiding the expulsion of the deutoplasm from the egg. Injuries to the posterior critical region where the embryo first develops have also to be carefully avoided. The eggs were kept in the fixative, washed for about two hours in 70% alcohol till all the picric acid is completely removed. Eggs thus treated can now be preserved in 70% alcohol with a few drops of glycerine for a long period.

For block making the eggs were dehydrated in graded series of alcohol. Considerable difficulty is experienced in sectioning these blocks due to hard chorion and excess of yolk material present, but this was overcome by using the technique of Slifer and King (1933) later modified by Roonwal (1936). Treatment of eggs with cupric phenol and subsequent soaking in water renders them soft for sectioning. Before passing the material to 90% alcohol, it was placed in 2% carbolic acid for about twenty four hours. Though Slifer has tried 4% carbolic acid but Roonwal's 2% carbolic acid was found entirely satisfactory for this purpose. Absolute alcohol was avoided as it hardens the material. Hardening and shrinkage

of the material can also be avoided at the time of clearing by using proper clearing agents. For this purpose several clearing agents like benzene, ceder-wood oil, amylacetate, xylol and carbo-xylol were tried. Only the last one proved to be the best of all. Neatly cleared material was transferred for two hours into pure molten paraffin wax kept in an incubator regulated at 58°C. Two bathes were given at one hour's interval.

The material was subsequently dropped with a warm needle into pure molten wax at the same temperature kept in a porcelin dish previously smeared with glycerine. The eggs were orientated with a warm needle under the binocular.

For sectioning the block were trimmed till the surface of the egg to be sectioned is fully exposed. Such blocks were soaked in water for about two to three days or more in order to further soften the chorion. Care was taken to start sectioning soon after soaking otherwise the material is likely to harden. All sections were cut at six to eight microns. Knife edge was kept scrupously sharp otherwise after a few sections they start crumbling. Entire series of sections was attached to slides smeared with albumin glycerine. After stretching on a hot plate the sections were dried for about two to three days in an incubator regulated at 40°C. Dried slides were passed through xylol, and then through descending grades of alcohol and finally washed in water.

Several stains were tried over the material like delafield's haematoxylin, acid-fuchsin, picro-nigrosin and iron-haematoxylin. Heidenhain's haematoxylin counter-stained with orange G or eosin were found most satisfactory. 3% iron alum was used as mordant and desired differentiation was obtained with 1-5% alum. Stained sections were cleared in benzene or xylol and mounted in D.P.X. Egg measurements were recorded from prepared slides and drawings were made with the help of camera lucida.

V. OBSERVATIONS ON FRESHLY LAID EGG

Mature female Chrotogonus lay their eggs in an egg-pod which is an elongated tubular structure made up of a frothy substance and gives protection to the eggs against injuries. Freshly formed egg-pod is light yellow which later changes into brown. Similar colour changes occur in the eggs within the pod. The egg-pod consists of two distinct parts. An anterior part which is plugged with froth and does not contain any egg, a posterior portion which is packed up with eggs exhibiting distinct arrangement. The entire egg pod is encrusted with sand particles. The eggs in the egg-pod are arranged in parallel rows with their longitudinal axis inclined at an angle to the vertical axis of the egg-pod. Each egg-pod contains 28-32 eggs, depending largely upon the duration of the egg laying. All egg-pods may not contain eggs as some of them were found to be empty. Similar observationshave been made by Pener and Shulov (1960) in Calliptamus palaestinensis. Individual eggs can be easily removed from the egg-pod which lie in moist soil, in dry soil the egg-pods shrivel up and become more or less brittle.

Freshly laid egg of Chrotogonus is a typically curved and elongated structure with the posterior end a little more bluntly rounded than the anterior. The shape of the individual eggs from the same egg-pod slightly varies. It is perhaps due

to close packing of fragile eggs in the egg-pod at the time of egg laying. The dorsal surface is convex while the ventral one is concave.

The colour of the egg is slightly yellow due to the yellow deutoplasm which shows through the translucent chorion. After one or two hours the colour changes to brown and in advanced stages of the development it picks up a dark-brown hue. Slight variation of colour also occurs in different regions of the same egg. At the posterior pole the colour is light green which later changes into brown. This change in colour starts from the posterior end and advances anteriorly till the entire egg turns dark brown in later stages of the development. The size of the egg is 3.06 mm in length and 1.43 mm in width. It is interesting to note that the egg shows remarkable change in size during development. It increases in the length as well as in the width. But the increase in width in the middle part of the egg is more pronounced than towards its length. Further, this increase is more prominent during the first half of the egg development but in later stages it is not as significant. This change in egg size is due to the absorption of water through the hydropylar pores from its surroundings during the course of development (Roonwal, 1936). On the basis of the above fact Roonwal considers the presence of water in the soil as one of the important factors, besides others, for a sustained development.

The entire egg is covered with a mucous membrane. The slippery nature of the membrane facilitates the passage of the egg through the genital tract. After oviposition the mucous layer becomes encrusted with sand particles. It degenerates during the course of development or may get destroyed by the micro-organisms present in the soil in which the egg pod is laid. Slifer (1937-1949) holds that since the membrane has no structural organisation and appears for a short while, it can not therefore be considered as one of the egg membranes. When removed, the surface of the underlying chorion becomes clearly visible. It's texture is quite rough and presents an sculptured appearance. The markings show a distinct mesh of hexagonal areas (Plate I, Fig. 1). Chorionic sculpturing is considered to be of practical importance as it makes identification of the eggs possible. It is believed to be produced by the impressions of the ovarian follicle cells upon plastic chorion as the egg passes through (Johnson & Butt, 1941), Slifer, 1937 and Hartley, 1961) et al.

The boundaries of hexagonal areas are brown in colour. Egg sculpturing is more compact at the posterior end than the anterior, in the middle region however the hexagonal surface is very distinct and forms a clean net work. In section the posterior region is sharply demarcated by this pattern which lends additional support in the identification of polarity of that egg. A crowding together of these areas give a distinctive brown colour to the posterior end which persist throughout

the entire development. The chorion is soft and thin in a newly laid egg but it gets hardened later causing fractures at different places at the time of emergence. The embryo comes out from the egg through anterior pole after rupturing the chorion in the form of a lid.

The posterior pole is characterised by the presence of micropylar canals and hydropylar apparatus. The micropylar canals form a complete ring of visible pores around the posterior end of the egg. The detailed structure of the micropyle, as described for Schistocerca (Husain & Roonwal, 1933) Locusta (Roonwal, 1954a,b and Jannone, 1939) in Dociostaurus, is essentially the same in Chrotogonus.

VI. STRUCTURE OF THE FIXED EGG

(1) The chorion and related structures:

The wall of a freshly laid egg is essentially similar in structure to that of a fully formed egg which has descended from the ovariole and is ready for oviposition. It consists of four layers which are arranged in the following order:

(i) An outer most refractile layer called extra-chorion. Underlying which is a thin contiguous, (ii) exo-chorion, (iii) a thick endo-chorion and finally (iv) the vitelline membrane.

The extra-chorion is a fragile, granular and slimy layer about 3-4 microns in thickness. It is composed of a mucous like substance to which a large number of sand grains adhere forming a rough surface. The extra-chorion seems to be loosely attached to the underlying layer (exo-chorion) as it can easily be removed by careful manipulation by a sharp needle. It goes into fragments as the layer dries up or is immerged in fixatives. In sections such fragment appear to bridge over the tubercles of the endo-chorion leaving spaces beneath (Plate I , Fig. 2,3. The hexagonal sculpturing of the chorion is faintly visible through the extra-chorion, but it could not be ascertained whether this layer is also sculptured the same way as the exo-chorion as reported by Uvarov

(1966). Husain & Roonwal (1933), Roonwal (1936a, 1954a) do not recognize any independent layer of extra-chorion in Schistocerca gregaria and Locusta migratoria migratorioides respectively. In Melanoplus differentialis Slifer (1937, 1949) also describes the extra-chorion as a mucoid coating over the chorionic surface which is sloughed off easily.

As regards the nature of extra-chorion Jahn (1935) considers it as a secretion of oviduct and not of the follicular epithelium in Melanoplus differentialis. Hartley (1961) has described the formation of this layer in Locusta migratoria migratorioides and observed that it appears after the egg has been separated from the follicular cells and can hardly be considered as a chorionic layer. Similar views are held by Uvarov (1966).

Underlying this layer is the chorion proper, divisible into the outer exo-chorion and an inner endo-chorion. The exo-chorion is a thin layer composed of fine granules which are less concentrated than those of endo-chorion. It is almost uniform in thickness all over the egg and measures about 7.38 microns. When stained with haematoxylin, it shows, in surface view, an indistinct hexagonal pattern. Each hexagon has a distinct central mass (Plate I, Fig. 1). In tangential sections it appears discrete, occupying space between two adjacent projections of the underlying endo-chorion (Plate I, Fig.2,3).

The endochorion is thick and is composed of two layers, an outer and an inner. The outer layer picks up a little more stain than the inner one and stands out in sharp contrast with the other. On the basis of differential staining it may be considered as denser than the outer one. The endochorionic surface is uneven due to the formation of tubercles - elongated, club-shaped projections from the outer layer of the endo-chorion. These tubercles are blind towards their free rounded ends. They differ in size, some are short and stumpy while a few are elongated and curved.

In the newly laid eggs the tubercles lie close together but with the advancing age they appear farther apart from each other in all parts of the egg except the posterior. In this region they are closely spaced. At the posterior end the inner layer of the endochorion also broadens considerably forming a concave disc-like structure. The tubercles do not penetrate the extra-chorion. The thickness of the endochorion in the middle of the egg is 10.3 microns, at the posterior end 14.5 microns.

Roonwal (1954a) contends that the short and elongated tubercles are nothing but fine extensions of the endochorion. These protuberances are reported to be in the body of the exo-chorion itself. Further he in his later paper (1954b) working on Schistocerca gregaria expresses the view that these tubercles are compact and are connected with their neighbours. Slifer

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(1937) describes two chorionic layers in Melanoplus differentialis an outer and an inner, the former less granular than the later. Jahn (1935) worked on the chemical nature of the two chorionic layers and contends that they are formed by the secretion of the follicular epithelium. Hartley (1961) discusses the formation of the two chorionic layers in Locusta migratoria. They are formed before the egg passes into the oviduct.

Below the endo-chorion lies a thin membrane - the vitelline membrane. The thickness of this membrane is slightly less than one micron. It envelops the deutoplasm and lies closely adpressed with the inner margins of the endo-chorion. Occasionally it detaches from contact with the endo-chorion due to fixation and subsequent manipulation and adheres to the yolk body at several places. The vitelline membrane remains intact during early development of the egg and disappears in about 60-hours old egg in which no trace of it could be seen in Chrotogonus.

Regarding the nature of this membrane there is a general unanimity of opinion that it is a structureless membrane (Johansen & Butt, 1941). The author while subscribing to this view failed to find any structure in this membrane. But Roonwal (1936) in Locusta migratoria migratorioides claims to have observed a definite structure of the vitelline membrane. He says, "it is not a structureless membrane, as has been maintained by all writers on insects, but exhibits, in a freshly

laid egg, a distinct felt-like structure. This is so in all Acrididae I have examined".

Roonwal (1954a) later working on the same insect arrived at a different conclusion by saying "next to the endochorion is the extremely thin (less than 1 u) and structureless vitelline membrane". A view in conformity with one generally upheld by embryologists. Further, the same author in (1936) describes a change from felt-like to a laminated structure superimposed over the former, and also a definite increase in thickness of the vitelline membrane of a 75-hours old egg. These changes persists till the embryo is about to hatch. This contention is again not substantiated in his work on Locusta migratoria migratorioides in (1958a) where, this membrane is claimed to have disappeared at the age 40½-hours. Slifer and King (1934) failed to find any such structure in Melanoplus and Chrotophaga eggs.

(2) The deutoplasm and the egg nucleus:

The deutoplasm or yolk which constitutes the main bulk of the egg is enclosed within the framework of the vitelline membrane. In the insect egg generally it is found in the form of spherules held in the meshwork of the protoplasm. Sections of the eggs of Chrotogonus show that the yolk spheres retain their original form at certain places near the periphery. The large bulk of yolk appears to have contracted and subsequently

split up into a number of irregular masses. This probably is due to the application of carbolic acid technique for softening the chorion. In these masses at certain places vacuoles are distinctly seen. Haematoxylin stains the yolk brown but eosin when used as a counter-stain changes this brown into voilet.

The protoplasmic layer forming the periplasm in the egg of insects belonging to different orders, particularly Lepidoptera, Hymenoptera and Diptera is not present in Chrotogonus. Similar conditions has been observed by Slifer & King (1934) in Melanoplus differentialis by Roonwal (1936b) in Locusta migratoria migratorioides and in Schistocerca gregaria by Jhingran (1949).

The process of maturation of egg has been worked out in considerable details by certain workers like Mc-Nabb (1928), Carother (1931), Slifer and King (1934), King and Slifer (1934) and Manna (1954). Among these the work of Mc-Nabb deserves special mention as he has beautifully illustrated the process of maturation in the eggs of grass-hoppers (Chrotophaga viridifasciata and Circotettix verrucculatus). Slifer and King, King and Slifer have also worked along the same line on fertilized and unfertilized eggs of Melanoplus differentialis. Carother has recorded observations on the maturation and segregation of heteromorphic homologus chromosomes in Acrididae. Manna has studied chromosomes during mieosis in fifteen species

of Indian grasshoppers. Mc-Nabb's observations may be summarised as follows:

In the freshly laid egg or in the egg which is about to be laid the metaphase stage of first maturation is observed. It has been further reported that the maturation process consists of two meiotic divisions. One of the first maturation telophase groups separates off into the first polar body and stays in the periphery, the other undergoes the second maturation division. With the completion of the second maturation division, the twelve monads in a group form the egg nucleus with two polar bodies. By the end of the process of maturation division the number of chromosomes in the egg nucleus is reduced to half the number that exists in the somatic cells. A similar reduction has occured in spermatogenesis and when the egg and sperm pronuclei unite in the process of fertilization, the resulting nucleus contains the diploid number of chromosomes which is the somatic number. The three polar bodies formed during the process degenerate. This fertilized nucleus with diploidy begins to cleave giving rise to two daughter cells by mitotic division.

Roonwal (1936) has described the early embryology of Locusta migratoria migratorioides but did not work out the process of maturation and fertilization and as such has accepted the work of Mc-Nabb, Slifer and King and King and Slifer. He starts the description of 4-cell cleavage stage at the age of 5½ hours.

In the longitudinal section of a freshly laid egg of Chrotogonus a certain pattern of distinctive chromosomes has been observed in the peripheral region of the posterior end almost towards the dorsal surface of the egg. It lies at a distance of 364 microns from the micopylar end (Plate I, Fig. 4, 5). The present author is inclined to infer that it is probably the metaphase of the first maturation division. But as sufficient material is not presently in hand it is proposed to work out the process of maturation in greater details at a later stage.

VII. CLEAVAGE, FORMATION OF BLASTODERM AND GERM-BAND

In a 10-hours old egg a few large cleavage cells are seen scattered in the peripheral region of the posterior end. These cells are six in number, somewhat rounded with a well marked centrally placed nucleus. The cytoplasmic part does not give rise to any process or extention which might connect two adjacent cells. The cells are almost identical in shape as in size and in their staining reaction. Further, these cleavage cells divide mitotically and later give rise to a large number of cells which begin to move towards the periphery (Plate I, Fig. 6). After some development has progressed it becomes impossible to count the exact number of dividing cells as the anterior end is cut off or punctured for proper penetration of the fixative through hardened chorion.

At about 24-hours the study of a longitudinal section shows that some of the dividing cells have migrated to the peripheral region and also towards the anterior pole (Plate I, Fig. 7). The process of migration has not been observed. It may probably be due to the centrifugal forces which exist in the developing egg as reported by Roonwal (1936). It could not also be ascertained whether the cleavage cell penetrate the yolk mass in order to reach the periphery as reported by Jhingran (1949).

The process of migration becomes pronounced as the cleavage progresses and the cells acquire definite shape. The cells which have migrated to the periphery are elongated compared to those somewhat rounded ones that lie at the anterior pole. The distance between two adjacent cells at the posterior pole is still almost the same in contrast to those which have migrated to the periphery. Sections of 24-hours show 28-32 cells in all, which are not connected by protoplasmic strands. The cytoplasm of the dividing cells also does not show such stellate processes which might connect two neighbouring cells.

In the region of the posterior end at about 30-hours the dividing cells are seen arranging themselves compactly in the form of a semicircular band. Apart from these large isolated cells in the band, other cells are also to be seen in the peripheral part (Plate II, Fig. 8). They are oval with elongated nuclei. It has been observed that the division rate which was slow till now has rapidly increased producing large number of cells. The mitotic spindles during division in the early stages do not show any definite orientation to the egg periphery - a fact which has been reported by different workers in the majority of the insects. The nuclei of the dividing cells at this stage do not show a distinct cytoplasmic coating over them as is evidenced by their staining reaction. Some cells show an early anaphase and telophase condition by their distinct chromosomal arrangement.

A primary epithelium is thus established under the chorion by peripheral migration of cleavage cells at about 42-hours of development. A longitudinal section of this stage (Plate II, Fig. 9) shows that all the cells comprising this epithelium are not of the same form or size in all regions of the egg.

A certain number of cells towards the posterior pole (numbering twelve in this particular longitudinal section) stand out distinctly by their large size, rounded form and close spacing from the rest. This part is considered here as the embryonic blastoderm (germ-disk). Since it gives rise to the germ-band from which the embryo proper shall be differentiated. The rest of the cells, greatly attenuated, are held at a distance from each other and constitute the extra-embryonic blastoderm. Their nuclei are elongated and stain less deeply with haematoxylin stain. The length of the cytoplasmic strands connecting these isolated nuclei is greatly variable, being shorter in the viscinity of the embryonic region than in more distant parts. Besides these cells, a few stellate cells with prominent nuclei are observed in the yolk mass. These are the vitellophages which could not be differentiated into primary and secondary yolk cells.

At about 48-hours the germ-disk epithelium undergoes rapid division resulting in the formation of oval cells which are compactly arranged in the form of a round concave disk in

the posterior region slightly on the ventral side of the egg (Plate II, Fig. 10, 12). The cells of the extra-embryonic primary epithelium are more or less spindle shaped with large nuclei.

The germ-band so formed begins to elongate along the ventro-anterior border of the posterior end. It becomes thick in the mid-region due to the accumulation of the dividing cells from the germ-disk. This thickening in the disk increases and ultimately acquires a multi-layered condition where the cells are arranged in two or three layers as seen in the egg of 58-hours (Plate II, Fig.11, 13).

VIII. EMBRYONIC MEMBRANES

(1) The amnion.

The formation of the two embryonic membranes - the amnion and serosa, in Chrotogonus follows the usual general pattern as exist in other insects. The two protective envelops appear simultaneously with the differentiation of the inner layer. As already described earlier, the germ-band at the age of 58-hours attains a multi-layered condition. It's lateral borders as seen in transverse sections now begin to invaginate into the yolk carrying along with them the part of the extra-embryonic blastoderm with which the extreme laterals parts of the germ-disk are continuous. A fold so formed is called the amniotic fold. These two folds which appear at the anterior and posterior ends at 68-hours begin to grow towards each other ventrad to the germ-band (Plate II, Fig. 14). The folds finally unite giving rise to two membranes, an inner called the amnion and the other outer - the serosa which is continuous with the extra-embryonic blastoderm. Between the amnion and germ-band a space is enclosed which is known as amniotic cavity.

(2) Serosa and its secretion.

As described earlier the serosa which forms a protective outer envelop appears concurrently with the amnion. The cells

of the serosa in the beginning are large with rounded nuclei and are connected by cytoplasmic strands. As development progresses further the serosal cells move apart from each other and become spindle-shaped. A single layered membrane thus envelops the developing embryo and the yolk mass.

In locusts and grasshoppers the serosa in addition to providing a protective membrane secretes a cuticular layer which gives support to the overlying chorion. The part of the serosa lying in the extreme posterior end shows a distinctive patch of cells of unknown function whose components lie in a single row. A patch similar in position yet more prominent containing cells distributed in a row has been reported in Melanoplus differentialis by Slifer (1938). This is recorded by him as a structure which helps in the absorption of water and has been called, hydropyler cells. Roonwal (1935) has observed a like structure in this very region and has termed it as, serosal patch. This is shown to be a double-layered structure with a space in between. No function has been assigned to it.

As soon as the serosa is differentiated it's cells secrete on their outer surface, below the endo-chorion, a thin cuticular layer, divisible into two distinct layers. A layer immediately in contact with the serosal cells does not stain with haematoxylin or eosin stain and is designated as white-cuticle. The layer of the cuticle lying between it and the

endo-chorion picks up yellow stain and is known as yellow-cuticle. These two layers become distinctly visible at 96-hours after the serosa has been fully established. The two cuticular layer appear simultaneously. With the secretion of this cuticular layer the chorion is so reinforced that sectioning becomes difficult and the carbolic acid treatment becomes necessary.

The inner surface of the yellow-cuticle in contact with the white-cuticle has a serrated margin. At the age of 96-hours both cuticular layers, yellow and white, attain a definite thickness. The thickness of the two cuticular layers is variable. The maximum variation is seen in the region of the posterior pole of the egg while in the rest of the egg the thickness is almost uniform measuring 7.38 microns (yellow cuticle) and 22.75 microns (white cuticle). In the region of the posterior pole the white cuticle increases, measuring 36.52 microns while the yellow cuticle measures 5.92 microns. It has been further observed that the white cuticle continues to increase pointing to the fact that the serosal cells remain active throughout the embryonic development (Plate I, Fig. 3 and Plate III, Fig. 15).

Roonwal (1954a,b) has described the formation of two cuticular layers in the African migratory locust Locusta migratoria migratorioides and in Desert locust Schistocerca gregaria respectively. He has pointed out that yellow cuticle

is secreted first followed by the white cuticle and the yellow cuticle does not show any serrations. Slifer (1937) has reported the formation of the cuticular layers in Melanoplus differentialis which are believed to be secreted at the same time. She has further reported that the margins of the yellow cuticle are serrated - an observation which accords with that of the present writer. The white cuticle is claimed to be digested before hatching by hatching enzymes. In addition to these two layers (yellow and white) she has reported another layer which is secreted by ordinary serosal cells and lies in contact with the white cuticle. The layer contains delicate striations at right angles to the surface. No such third cuticular layer is seen in Chrotogonus. Matthee (1951) reported the formation of two layer (white and yellow) in Locustana pardalina. This yellow cuticle never becomes as thick as the white but has the same structure and stains the same way as that in Melanoplus differentialis (Slifer, 1937).

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X. LIST OF ABBREVIATIONS

amca	=	amniotic cavity
amf	=	amniotic fold
ch	=	chorion
cn	=	cleavage nucleus
extch	=	extra-chorion
exch	=	exo-chorion
ench	=	endo-chorion
exemb	=	extra-embryonic region
emb	=	embryonic region
gb	=	germ-band
n	=	nucleus
P	=	Posterior pole
cs	=	cytoplasmic strand
ser	=	serosa
serc	=	serosal cell
vm	=	vitelline membrane
wcu	=	white cuticle
ycu	=	yellow cuticle
y	=	yolk
yc	=	yolk cell
pserc	=	posterior serosal cells

EXPLANATION OF PLATES (I-III)

PLATE - I

EXPLANATION OF FIGURES

Fig. 1. Tangential section of chorion, showing chorionic sculpture and hexagonal pattern of chorion.

Fig. 2. Longitudinal section of egg wall of freshly laid egg, showing the vitelline membrane and chorionic layers.

Fig. 3. Longitudinal section of egg wall of mid-region at 96-hours, showing the serosal cell, white and yellow cuticle.

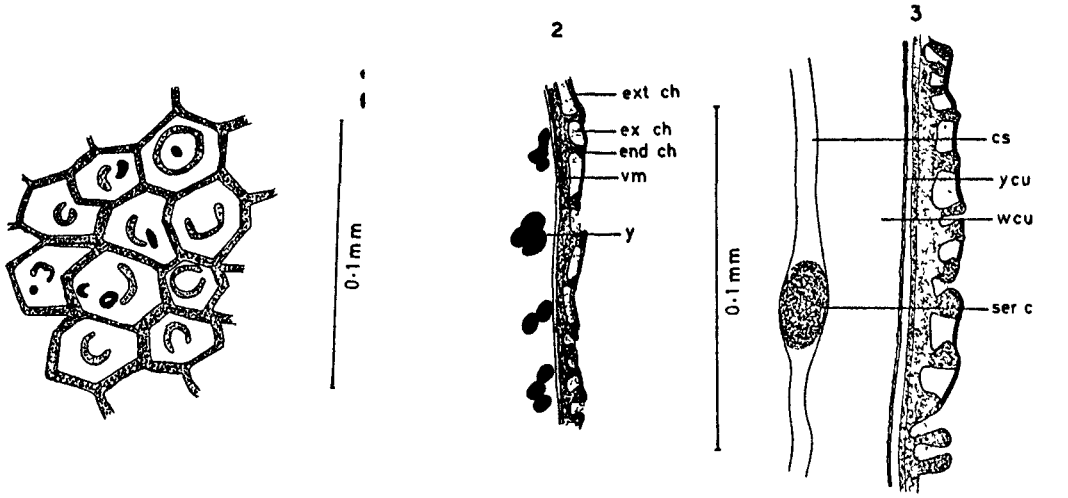
Fig. 4. Longitudinal section of posterior end of a freshly laid egg showing the yolk masses and position of the egg nucleus.

Fig. 5. A portion of the posterior pole of a freshly laid egg, showing the chromosomes complex.

Fig. 6. Longitudinal section of posterior end at 10-hours, showing six cleavage cell stage.

Fig. 7. Longitudinal section of posterior end at 24-hours, showing the arrangement of cleavage nuclei on the periphery.

PLATE-I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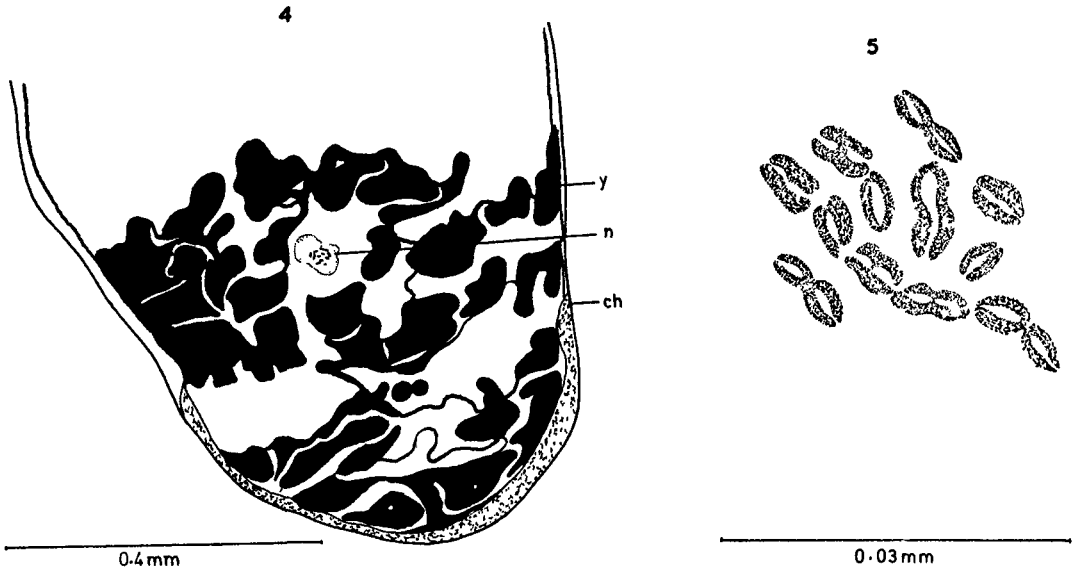
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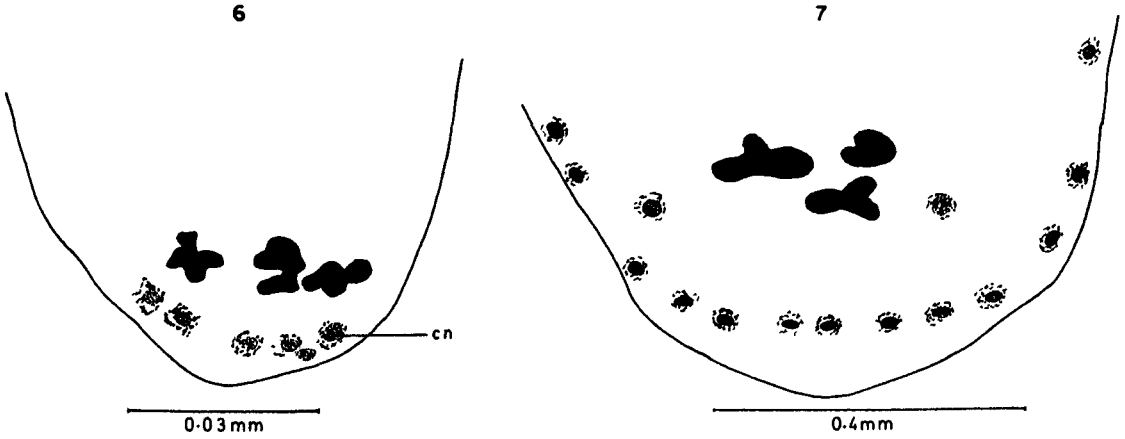
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PLATE - II

EXPLANATION OF FIGURES

Fig. 8. Transverse section across the posterior end of an egg, showing a semi-circular band of dividing cells - 30 hours.

Fig. 9. Longitudinal section of posterior end at 42-hours, showing the embryonic region (blastoderm) and extra-embryonic region.

Fig. 10. Longitudinal section of posterior end at 48-hours, showing the germ band and extra-embryonic region.

Fig. 11. Longitudinal section of a posterior end at 58-hours, showing the multi-layered condition of germ disk.

Fig. 12. Transverse section across the posterior end at 48-hours, showing the double layered condition of germ disk.

Fig. 13. Transverse section across the posterior end at 58-hours showing the multi-layered condition of germ band.

Fig. 14. Transverse section across the posterior end at 68-hours, showing the two amniotic folds and amniotic cavity.

PLATE–II

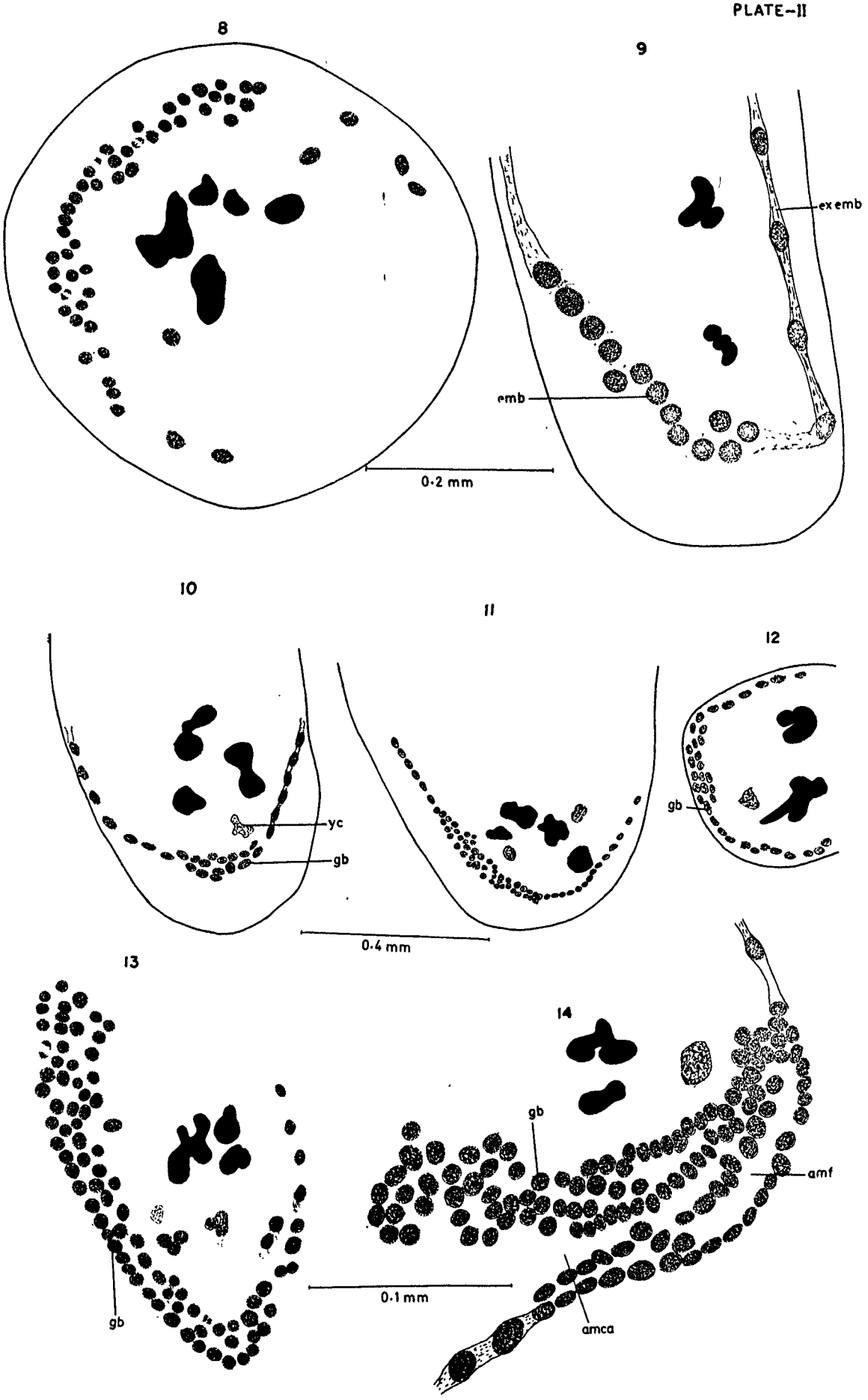

PLATE - III

EXPLANATION OF FIGURES

Fig. 15. Longitudinal section of posterior end at 96-hours, showing posterior serosal cells.

PLATE-III

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